applied sciences

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Valorization of Cassava By-Products: Cyanide Content and Quality Characteristics of Leaves and Peel

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Abstract: Cassava production generates significant amounts of by-products such as leaves and tuber peel. Instead of considering them as waste, valorization aims to find sustainable ways to utilize them. However, the presence of cyanide and insoluble fibers poses a major obstacle to their conversion into valuable products. Therefore, the objective of this study is to investigate the changes in cyanide concentration and quality of cassava leaves after mechanical pressing and in tuber peel after treatment with an enzyme solution. Frozen leaves were screw-pressed into their fractions: juice, and press cake. The results show that the cyanide level in the press cake was reduced to 73.56% and was concentrated by 97.48% in the juice compared to the frozen leaves. However, the crude protein values of the frozen leaves, juice, and press cake did not differ significantly ($p > 0.05$), and were 27.09%, 25.47%, and 23.82%, respectively. In addition, the results for the peel revealed that pretreatment with Viscozyme® L, which assists in the mechanical peeling of cassava tubers, also contributed to a reduction in cyanide and insoluble fiber in the peel. Cyanide content was lowered by 53.89–58.94% in enzyme-treated peel from all three runs (ETP1-3) when compared to fresh peel (FP), while the reduction was only 8.63% in the control peel (CP) treated with hot water without enzyme solution. The insoluble fibers in cassava peel, such as neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fiber (CF), were also degraded more effectively after treatment with an enzyme solution than with hot water.

Keywords: cyanogenic glycosides; crude protein; leaf fractions; mechanical peeling; insoluble fiber

Citation: Mukhtar, A.; Latif, S.; Barati, Z.; Müller, J. Valorization of Cassava By-Products: Cyanide Content and Quality Characteristics of Leaves and Peel. *Appl. Sci.* **2023**, *13*, 6340. https://doi.org/10.3390/app13106340

Academic Editors: Vidal Natalia Prieto and Emmanouil Tsochatzis

Received: 18 April 2023
Revised: 16 May 2023
Accepted: 20 May 2023
Published: 22 May 2023

1. Introduction

Cassava (*Manihot esculenta*) is an important food crop that is widely cultivated in numerous regions of the world, mainly in the tropics and subtropics, with an estimated production of more than 250 million tons [1]. It is known by various names such as yucca in Latin America, manioc in Brazil, and tapioca in Southeast Asia. Cassava is a versatile crop that can be grown in a variety of soil types and can cope with drought and other environmental stresses, making it a valuable crop for food security. Recently, increasing attention has been given to cassava farming in other regions of the globe due to its greater adaptability. Cassava is grown primarily for its starchy root as it is a major source of carbohydrates for millions of people, especially in regions where other staple foods are not readily available. However, its by-products such as leaves and tuber peel are usually disposed of as waste, which ultimately pollutes the environment. To date, several studies have been conducted to determine the nutritional composition and potential benefits of cassava leaves [2–6] and peel [7–13]. In most of these studies, it has been well documented that cassava leaves are a rich source of protein and other valuable nutrients such as vitamins, minerals, and antioxidants [1,14]. They can be used as a food source for humans or as animal feed. Cassava peel, on the other hand, is a good source of fiber and can be used as

animal feed, in paper production, and as biodegradable packaging. In addition, cassava peel can also be processed to produce ethanol as a renewable energy source, which can help reduce reliance on fossil fuels and promote sustainable development. Thus, the efficient utilization of cassava by-products can help reduce waste and improve the sustainability of cassava production.

Despite the great potential of cassava leaves and peel, their use is limited due to their high levels of antinutritional components, such as cyanogenic glycosides, which can be harmful to human health if not properly detoxified [15]. Cyanogenic glycosides occur in cassava tissue in three forms, linamarin and lotaustralin, cyanohydrin, and free cyanide. Linamarin is the predominant form, accounting for 93% of the total cyanogenic glycosides present in cassava [16]. Linamarin is hydrolyzed to glucose and acetone cyanohydrin in the presence of linamarase. Acetone cyanohydrin is then spontaneously degraded to HCN and acetone by hydroxynitril lyase (HNL) [17]. Linamarin is usually located in the vacuole, while linamarase and HNL are localized in the cell wall [18]. Various processing methods such as drying [19–21], cooking [20,22,23], fermentation [2,10,19], enzyme and ultrasonic treatment [24], and membrane filtration or coagulation [25] have been well studied in relation to the removal of cyanide from cassava tissues of different varieties. However, most detoxification methods usually require 15 min of pounding followed by 10 to 120 min of boiling and washing with tap water. Undoubtedly, the proposed methods can reduce cyanide levels in cassava tissue to well below the FAO/WHO safe limit of 10 ppm [26]. However, these methods require long processing times, and a lot of water is wasted during washing, resulting in the loss of valuable nutrients.

Considering the previous efforts, a mechanical screw press was used for this study to separate the cassava leaves into their fractions: juice and press cake. The processing of cassava leaves by screw presses not only helps to obtain a press cake with concentrated protein but also reduces the cyanide content by leaching the cyanide with the juice fraction [3]. Some researchers have already used fresh leaves to explore the properties of cassava leaves and their products after screw pressing [21,27]. However, in our experiments we used frozen cassava leaves because freezing is a fast, convenient, and popular method for food preservation with minimal nutrient loss. In addition, freezing leads to various structural changes in the fresh leaves, such as cell lysis, cracking, and dislocation [28], which could increase the availability of proteins for extraction and may also have a positive effect on the leaching of cyanide in the juice fraction during pressing. Moreover, frozen leaves could be readily available throughout the year.

In the case of cassava peel, in addition to cyanide, the presence of insoluble fiber fractions, such as neutral detergent fiber (NDF) which includes cellulose, hemicellulose, and lignin; acid detergent fiber (ADF) comprising lignin and cellulose; and acid detergent lignin (ADL) consisting of lignin, poses a great challenge in processing. Under natural conditions, the hemicellulose and lignin form a protective matrix around the cellulose, which interferes with the digestion of the peel [29]. Therefore, it is necessary to hydrolyze the insoluble components of the peel so it can subsequently be used as feed or converted into other usable products. The peeling methods employed can also affect the insoluble fiber fractions by modifying the peel's composition and structural properties. Various methods, such as manual, mechanical, chemical, and thermal methods, are currently used to remove the peel from the tubers. However, despite the fact that mechanical peeling is widely used in both small- and large-scale industries because of its simplicity and speed, it results in a significant loss of flesh of about 25–44% [30,31]. To address this problem, Barati et al. [7] discovered that enzyme treatment prior to mechanical peeling can increase the efficiency of the peeling process by softening the cell wall components of the peel. This study is an extension of previous research and aims to determine if enzyme-assisted mechanical peeling can enhance the hydrolysis of insoluble fibers in cassava peel. Additionally, this study examines whether enzyme treatment prior to mechanical peeling could also contribute to the detoxification of cassava peel by promoting contact between linamarin and endogenous linamarase [32]. Thus, overall, this study had the following

objectives: (1) To explore the influence of mechanical pressing on the composition, protein, and cyanide levels of frozen cassava leaf fractions: juice, and press cake. (2) To examine the effects of enzymatic pretreatment on the composition, insoluble fiber, and cyanide content of cassava tuber peel.

2. Materials and Methods

2.1. Cassava Leaves and Their Fractions

Approximately 2 kg of frozen, chopped cassava leaves were purchased in two batches from an African shop in Stuttgart, Germany. The leaves were stored at −20 °C and thawed at room temperature before being used for the experiments. Screw pressing was performed separately for each batch by using a commercial laboratory scale stainless steel twin-gear screw press (AG-8500S, Angel Juicers, Toogoolawah, QLD, Australia). A coarse mesh screen with a hole size of 1 mm was installed in the press and the processing was conducted at room temperature with a screw speed of 82 rpm. The screw press was continually fed to separate the juice and press cake, as shown in Figure 1. The samples were packed and stored at −20 °C until further analysis. Each batch was analyzed at least in triplicate, and the findings of both batches are reported together.

Figure 1. Screw pressing of cassava leaves into their fractions: juice, and press cake.

2.2. Enzymatic Pretreatment for Peeling Cassava Tubers

Fresh cassava tubers were obtained from a local market in Stuttgart, Germany, and stored in a refrigerator at 10 °C for the experiments. The tuber peel was removed using the method described as optimal by Barati et al. [7], which includes three main steps: (1) application of meridian incisions, (2) enzymatic pretreatment, and (3) mechanical peeling. For the meridian incisions, three tubers of mixed sizes: large, medium, and small, were first incised by a sharp knife at intervals of about 10 mm to a depth of almost 4 mm to facilitate the infusion of the enzyme to hydrolyze the cassava peel. For enzymatic treatment, Viscozyme® L (Novozymes GmbH, Essen, Germany), which is a mixture of beta-glucanases,

pectinase, hemicellulase, and xylanase, was used. After incising, the cassava tubers were immersed in Viscozyme® L solution containing an enzyme dose of 1.2 mL g^{-1} of peel, corresponding to about 18% of the mass of the cassava tuber, with 5 L of distilled water at a temperature of 50 °C, a pH of 4.5, and an incubation time of 4.5 h. After enzyme treatment, the treated cassava tubers were peeled using a prototype peeling machine at a speed of 172 ± 1 rpm for 3 min. After completion of the first run, the enzyme solution was allowed to cool at room temperature and stored in the refrigerator at 10 °C. The same enzyme solution was used for the second and third runs on the following days without any modification. The peel obtained from each of the three runs was named enzyme-treated peel (ETP1-3). In order to have a comparison, water without Viscozyme® L solution was used as a control under the same conditions. The entire experiment was performed in duplicate. All peel samples, including fresh peel (FP), control peel (CP) treated with hot water without any enzyme solution, and enzyme-treated peel (ETP1-3) were stored at −20 °C for further analysis. The experimental setup is shown in Figure 2 to allow for a better understanding of the process.

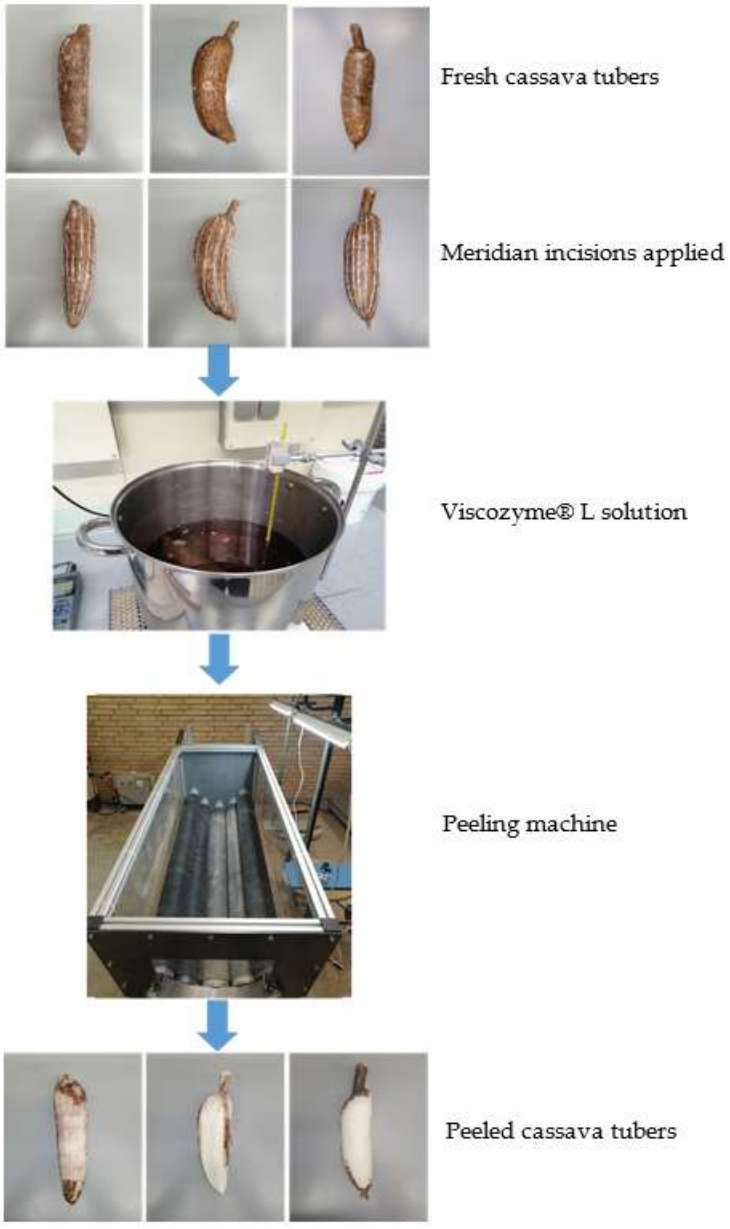

Figure 2. Viscozyme® L pretreatment and mechanical peeling of cassava tubers.

2.3. Analysis of Dry Matter, Organic Matter, Crude Protein, and Fiber

The dry matter of cassava juice, press cake, and leaves was measured by drying the samples at 105 °C for 12 h [33]. Ash and organic matter were determined by heating the oven-dried samples in a muffle furnace according to AOAC Official method 923.03 [33]. To measure the crude protein of the cassava leaves, the Kjeldahl method and the Kjeldahl analysis system (Vapodest 500, C. Gerhardt GmbH & Co. KG., Königswinter, Germany) were used. This process involves three main steps: digestion, distillation and titration. First, the sample was digested in the presence of concentrated sulfuric acid, which converts organic nitrogen to ammonium sulfate. Then, the digested sample was distilled in the presence of an alkali, which released the ammonia gas from the ammonium sulfate. Finally, the ammonia gas was titrated with a standardized acid solution, and the nitrogen content of the sample was calculated based on the amount of acid used in the titration. The total nitrogen content was converted to crude protein using a conversion factor of 6.25. The neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fiber (CF) of the cassava peel were examined according to official AOAC method 973.18 (FiberBag Analysis System FBS6, C. Gerhardt GmbH & Co. KG., Königswinter, Germany) [33]. In this method, the cassava peel was first ground and then placed in a FiberBag, which is made of a porous material that allows the solutions to penetrate the sample. The FiberBag was then placed in the FBS6 system, which performed the entire analysis automatically. Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fiber (CF) were measured separately. NDF is the fraction of fiber that is insoluble in neutral detergents, ADF is the fraction of fiber that is insoluble in acid detergents, ADL is the fraction of ADF that consists of acid-insoluble lignin, and CF is the residual material after extraction of the fibers with acid and alkali. All measurements were performed at least in triplicate.

2.4. Cyanide Measurement

The picrate paper method was used to measure the total amount of cyanide in cassava leaves, juice, press cake, and peel [34]. For this, the first picrate paper was prepared by dipping 0.3 mm thick filter paper in a 14% (w/v) picric acid solution (Sigma-Aldrich, St. Louis, MO, USA) and drying the paper in a fume hood. The dried paper was then cut into a 3 cm × 1 cm rectangle and joined with a 5 cm × 1 cm plastic strip that was 1 mm thick. Linamarase was extracted according to the method developed by Haque and Bradbury [35]. In a vial, approximately 0.15 g of the sample, 1 mL of 0.1 M sodium phosphate buffer, and 100 µL of linamarase were combined. A plastic strip prepared with the picrate paper was placed inside the vial, which was then sealed with a screw cap and mixed thoroughly before being stored at 30 °C for 24 h. During the incubation, the picrate paper remained in the vial without direct contact with the liquid. After the incubation, the picrate paper was taken out and soaked in 5 mL of distilled water for 45 min. For comparison, a blank picrate paper, suspended in a vial without a sample, was used. To establish a standard curve for cyanide content, various concentrations of linamarin (Sigma-Aldrich) ranging from 0.2 µM to 1.4 µM were used. Both the blank and standard picrate papers were treated similarly to the sample picrate papers. Finally, the absorbance of the solution was then assessed at 510 nm against water.

2.5. Statistical Analysis

The statistical software IBM SPSS 22.0 was used to perform the statistical analysis. The ANOVA and Tukey's honestly significant difference (HSD) tests were used to detect mean differences between treatments at a significance level of $\alpha = 0.05$. Data were plotted using Origin Pro 2020 (Origin Lab Co., Northampton, MA, USA).

3. Results and Discussion

3.1. Quality Assessment and Cyanide Level of Cassava Leaves following Screw Pressing

The results of screw pressing frozen cassava leaves reveal the unequal distribution of the original ingredients between their fractions: juice and press cake. Figure 3 shows that the press cake contains a high proportion of dry matter and organic matter compared to the juice fractions, indicating that press cake can be used in the production of high-quality silage. However, the ash content was higher in the juice part than in the press cake. This is due to the fact that some minerals were leached during the pressing process, resulting in a greater amount of ash in the juice. These findings are consistent with those of Latif et al. [27], who observed a similar trend during the mechanical pressing of cassava leaves. As for crude protein content, a reasonable amount of protein was found in the frozen leaves (27.09% DM), as well as in the juice (25.47% DM) and press cake (23.82% DM). The protein value of the juice fraction was higher than that of the press cake, which is in contrast to the results found by other researchers [3,27] who reported a high protein content in the press cake. This difference could be explained by the fact that most of the proteins in leaves are bound in the fibrous structure [36], which typically leads to a higher protein content in the press cake during mechanical pressing. However, in the present study, frozen leaves were used, and freezing as a pretreatment resulted in cell breakage and the release of cell components [28], including soluble protein, leading to a high protein content in the juice fraction. Despite this difference, there was no significant distinction in the protein content of frozen cassava leaves and their fractions, suggesting that they have great potential for protein extraction for use as food or feed. Several researchers have identified the related amount of protein in cassava leaves from different varieties of the plant. For instance, Leguizamón et al. [14] reported protein content ranging from approximately 19 to 25% DM in three cassava varieties. In another study, Oni et al. [37] obtained protein concentrations in cassava leaves from four varieties ranging from 17.7% to 24% DM. Ayele et al. [21] found a protein content of 33.6% DM in young cassava leaves. However, it should be noted that the quality and quantity of protein in cassava leaves and their fractions may vary due to differences in variety, harvest age, climatic conditions, soil properties, freezing or storage time, pressing, and extraction methods.

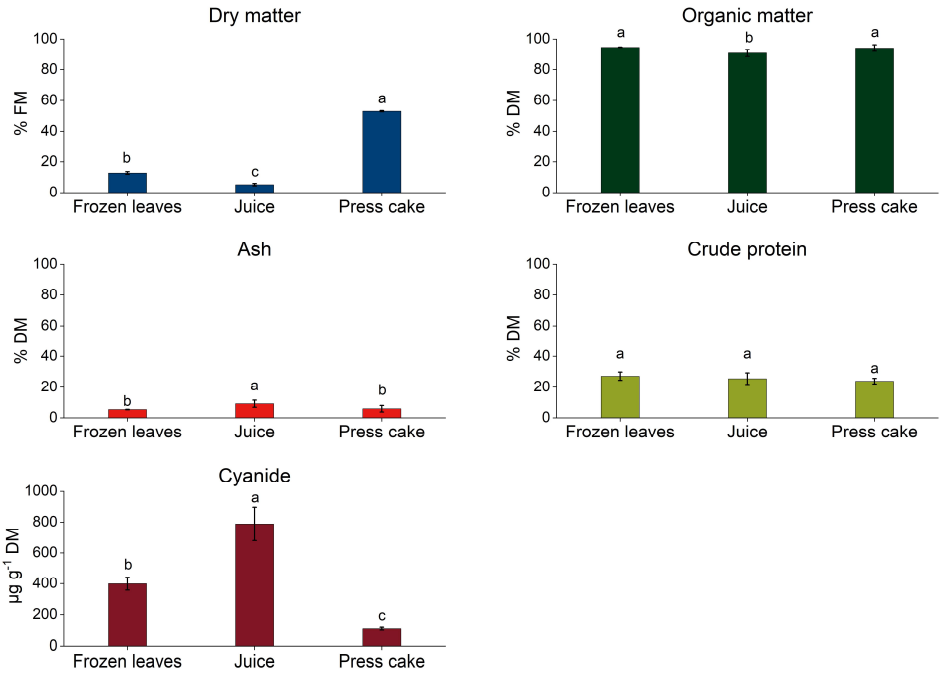

Figure 3. Dry matter, organic matter, ash, crude protein, and cyanide content of frozen cassava leaves, juice and press cake. Different letters for the same parameter represent significant differences ($p < 0.05$).

The cyanide results in Figure 3 show that the initial cyanide content of 399.23 μg g^{-1} DM in the frozen leaves decreased to 105.55 μg g^{-1} DM in the press cake, but increased to 788.38 μg g^{-1} DM in the juice portion. This indicates that most of the cyanide content was removed with the juice part during the crushing process, and pressing alone was effective in obtaining a press cake with low toxicity. Moreover, freezing and thawing prior to pressing causes a disruption in leaf tissue and cells, which may also contribute to a greater reduction in cyanide by releasing the bound cyanide and facilitating the interaction of linamarin with endogenous linamarase. Ogbadoyi et al. [38] reported that freezing tissues with a high moisture content causes the formation of ice crystals within the cells and this ruptures the cell membranes, leading to a leakage of cells and their contents. Since cyanide is water-soluble, it can be trapped in the ice crystals and released upon thawing, and thus is easily leached out with the juice fraction during pressing. These findings confirm that simple screw pressing of frozen leaves is a promising method to obtain a press cake with considerably lower cyanide content. While some researchers have reported on the screw pressing of fresh cassava leaves, the pressing of frozen cassava leaves remains relatively unexplored, adding further novelty to this research. However, even after pressing, the cyanide level in the press cake is still above the safe limit of 10 ppm set by the FAO/WHO [26]. Therefore, to reduce the additional cyanide content in the press cake and make it valuable for use as food or feed, further processing is required. In contrast, the juice fraction can be used to extract linamarin or HCN, which have numerous industrial applications including pesticides, chemical synthesis, dyes, synthetic fiber production, plastics, mining, electroplating, and fumigation.

3.2. Changes in Composition, Fiber, and Cyanide Content of Cassava Tuber Peel after Enzymatic Treatment

In Figure 4, the compositional analysis of cassava tuber peel reveals some changes after treatment with hot water and Viscozyme® L solution. Dry matter and organic matter were higher in the fresh peel (FP) and the enzyme-treated peel (ETP1-3) than in the control peel (CP) treated with hot water without enzyme solution. However, the ash content in the CP was high, which can be directly attributed to the low organic matter. Insoluble fibers, including neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fiber (CF), were significantly reduced in both the CP and the ETP1-3. The initial NDF value in the FP of 61.01% DM decreased to 35.13% DM for the CP, and to 33.18–33.16% DM for the ETP1-3, but no significant change was observed in the NDF content of cassava peel after treatment with hot water and enzyme solution. The ADF content of the ETP1-3 was 24.01–28.91% DM, which was lower than that of the CP, 31.55% DM, and the FP, 57.00% DM. This means that enzymatic treatment is more effective than hot water treatment in the hydrolysis of ADF. The lowest ADL value of 10.43% DM was obtained for the ETP1, while no significant difference was found for the CP and the ETP2 and the ETP3. This could be due to the fact that after the completion of the first run (ETP1), the same enzyme solution was used for the ETP2 and the ETP3 in the following days. It might be possible that some enzymes were degraded during the first run and storage, resulting in a decrease in the efficiency of ADL hydrolysis for the ETP2 and the ETP3. The crude fiber (CF) content in the FP of 35.01% DM was significantly reduced to 14.36–14.40% DM for both CP and ETP1-3. Overall, both hot water and enzyme treatments were successful in hydrolyzing the insoluble fibers (NDF, ADF, ADL and CF) of cassava peel; however, enzymatic treatment was found to be more effective in hydrolyzing ADF and ADL. Similar results were also discussed by other researchers, who reported the positive effects of amylase, cellulase, and pectinase enzymes under different optimal conditions for the hydrolysis of lignocellulosic materials [39–41]. According to Olaniyi et al. [42], the crude fiber content of cassava peel and corn cobs subjected to enzyme treatment was reduced by 13.75% and 29.70%, respectively. Many studies have discussed the role of various enzymes in the hydrolysis of cassava peel. However, in this study Viscozyme® L was used and the conditions identified by Ziba et al. [7] as optimal for the mechanical

peeling of cassava tubers also showed a positive effect on the hydrolysis of insoluble fibers. Therefore, enzymatic pretreatment prior to mechanical peeling could improve the potential for the further use of cassava peel as animal feed or for converting complex carbohydrates to simple sugars through fermentation.

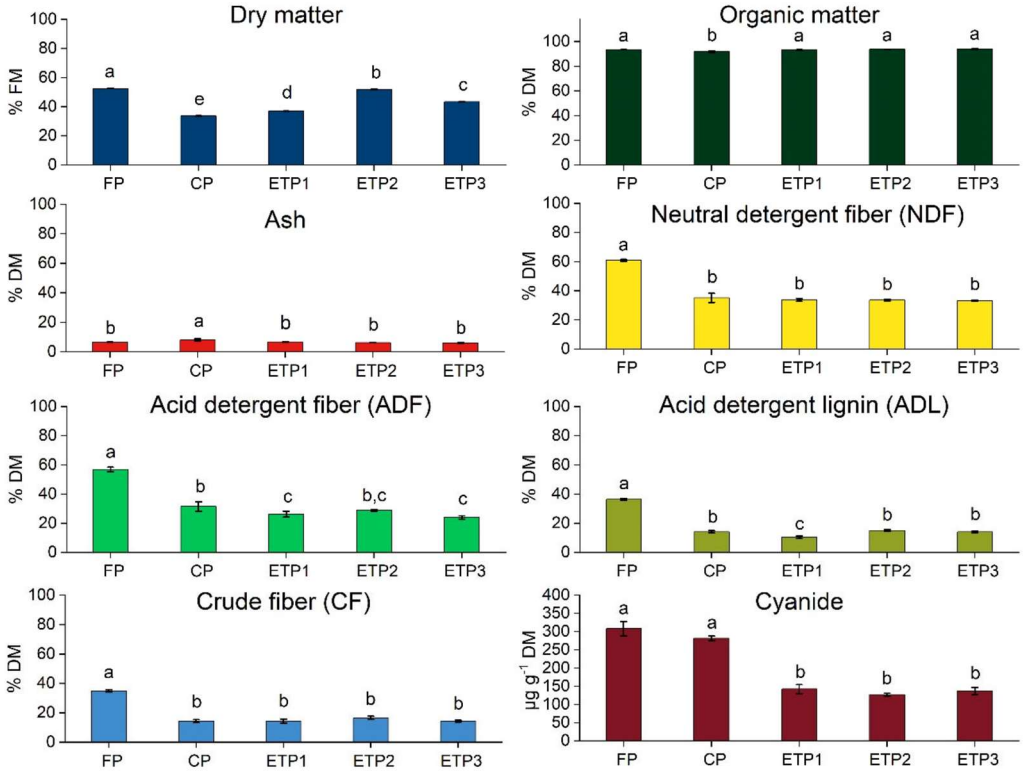

Figure 4. Dry matter, organic matter, ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), crude fiber (CF), and cyanide content of fresh cassava tuber peel (FP), control peel (CP) treated with hot water without enzyme solution, and enzyme-treated peel (ETP1-3) obtained after three separate runs with the same enzyme solution. Different letters for the same parameters indicate significant differences ($p < 0.05$).

Regarding cyanide content, the findings reveal that the enzyme-treated peel (ETP1-3) had a significantly lower cyanide content of 126.61–142.17 µg g^{-1} compared with the cyanide content of the fresh peel which was 308.35 µg g^{-1} and that of the control peel treated with hot water which was 281.75 µg g^{-1}. The significantly lower cyanide content indicates that enzyme treatment prior to mechanical peeling is more effective in reducing cyanide concentration than hot water treatment. This is due to the ability of the enzyme to hydrolyze, break, and damage the cell wall [43]. Once the cell wall is damaged and cells are disrupted, this does not only promote the release of endogenous linamarase and trapped linamarin, but also accelerates the degradation of linamarin, as reported by Sornyotha et al. [32] and Estiasih [18].

While there is considerable information in the literature about the role of enzymes in the hydrolysis of cassava peel and the reduction of cyanide content, this research introduces several novel aspects. Firstly, this study specifically focuses on the use of Viscozyme® L, which has not been previously employed for this purpose. In a previous study, we successfully identified its role in the mechanical peeling of cassava tubers. Building upon that, this study further explains its ability to reduce cyanide content and hydrolyze insoluble fibers. Additionally, this study highlights the possibility of reusing the same Viscozyme® L solution in multiple cycles, which has not been reported before. This demonstrates the potential for reusing the enzyme solution and optimizing its efficiency in cyanide reduction.

4. Conclusions

The results show that screw pressing of frozen cassava leaves can produce a press cake with a significantly low cyanide content and a high protein content, which supports the use of screw presses in cassava leaf processing. However, in order to use the press cake as food or feed, further reduction of cyanide content below the FAO/WHO permissible limit of 10 ppm is required. Moreover, the juice fraction with the higher cyanide content can be further processed to obtain linamarin or HCN that can be used in various industrial applications. In addition, the study reported that the pretreatment of cassava tuber peel with Viscozyme® L prior to mechanical peeling showed a positive effect in terms of significantly reducing the cyanide content and hydrolysis of insoluble fibers in the cassava peel. This implies that the use of an enzyme solution in mechanical peeling not only has benefits in relation to peeling but also contributes to the better processing of cassava peel waste. The promising results of using the same enzyme solution in three consecutive runs indicate the possibility of reusing the enzymes in multiple runs. Therefore, future research should focus on determining the enzyme activity remaining after each run or adding a specific amount of enzyme that could be degraded during the process to make it more cost-effective while maintaining the efficiency of the peeling process.

Author Contributions: Conceptualization, A.M., S.L., Z.B. and J.M.; methodology, A.M., S.L. and Z.B.; software, A.M.; validation, A.M., S.L., Z.B. and J.M.; formal analysis, A.M., S.L., Z.B. and J.M.; investigation, A.M. and S.L.; resources, A.M., S.L., Z.B. and J.M.; data curation, A.M. and S.L.; writing—original draft preparation, A.M.; writing—review and editing, A.M., S.L., Z.B. and J.M.; visualization, A.M., S.L., Z.B. and J.M.; supervision S.L., Z.B. and J.M.; project administration, A.M., S.L. and J.M.; funding acquisition, S.L. and J.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fiat Panis Foundation (Ulm, Germany) grant number 10/2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting this study are available on request from the corresponding authors.

Acknowledgments: The authors would like to thank the lab team of the Institute of Agricultural Engineering (440e) Tropics and Subtropics Group for analyzing the samples and Sabine Nugent for the English proofreading of this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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